The Relationship between Environmental Exposures to Phthalates and DNA Damage in Human Sperm Using the Neutral Comet Assay

Susan M. Duty,[1] Narendra P. Singh,[2] Manori J. Silva,[3] Dana B. Barr,[3] John W. Brock,[3] Louise Ryan,[4] Robert F. Herrick,[1] David C. Christiani,[1,5] and Russ Hauser[1]

[1]Department of Environmental Health, Occupational Health Program, Harvard School of Public Health, Boston, Massachusetts, USA; [2]Department of Bioengineering, University of Washington, Seattle, Washington, USA; [3]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [4]Department of Biostatistics, Harvard School of Public Health, and Department of Biostatistical Science, Dana-Farber Cancer Institute, Boston, Massachusetts, USA; [5]Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA

Phthalates are industrial chemicals widely used in many commercial applications. The general population is exposed to phthalates through consumer products as well as through diet and medical treatments. To determine whether environmental levels of phthalates are associated with altered DNA integrity in human sperm, we selected a population without identified sources of exposure to phthalates. One hundred sixty-eight subjects recruited from the Massachusetts General Hospital Andrology Laboratory provided a semen and a urine sample. Eight phthalate metabolites were measured in urine by using high-performance liquid chromatography and tandem mass spectrometry; data were corrected for urine dilution by adjusting for specific gravity. The neutral single-cell microgel electrophoresis assay (comet assay) was used to measure DNA integrity in sperm. VisComet image analysis software was used to measure comet extent, a measure of total comet length (micrometers); percent DNA in tail (tail%), a measure of the proportion of total DNA present in the comet tail; and tail distributed moment (TDM), an integrated measure of length and intensity (micrometers). For an interquartile range increase in specific gravity–adjusted monoethyl phthalate (MEP) level, the comet extent increased significantly by 3.6 μm [95% confidence interval (95% CI), 0.74–6.47]; the TDM also increased 1.2 μm (95% CI, –0.05 to 2.38) but was of borderline significance. Monobutyl, monobenzyl, monomethyl, and mono-2-ethylhexyl phthalates were not significantly associated with comet assay parameters. In conclusion, this study represents the first human data to demonstrate that urinary MEP, at environmental levels, is associated with increased DNA damage in sperm. *Key words:* **comet assay, DNA damage, environmental, human sperm, phthalates, urinary metabolites.** *Environ Health Perspect* **111:1164–1169 (2003). doi:10.1289/ehp.5756 available via** *http://dx.doi.org/* **[Online 6 December 2002]**

Phthalates are multifunctional chemicals used to hold color and scent in consumer and personal care products (Koo et al. 2002); as carpet backing and as solvents in paints, glue, and insect repellents (ATSDR 1999); and to soften a wide range of plastic goods (Bradbury 1996). Di(2-ethylhexyl) phthalate (DEHP), one of the more commonly used phthalates, leaches from blood products, intravenous and dialysate bags, and tubing made with polyvinyl chloride (Nässberger et al. 1987). Phthalates are also present in drinking water, air, and food (ATSDR 1995, 1999, 2000). Despite the rapid metabolism and elimination of most phthalates (Koo et al. 2002; Nässberger et al. 1987; Peck and Albro 1982), theoretically a constant steady state may be reached because of chronic and repetitive, low-level exposures from dietary ingestion and from many commonly used products.

Evidence of widespread exposure of the U.S. population to phthalates comes from two recent studies on the levels of phthalate metabolites in urine samples collected for the Third National Health and Nutrition Examination Survey (NHANES III) (Blount et al. 2000b) and NHANES 1999 (CDC 2001). The NHANES surveys collect biological samples and information about the health and diet of people in the United States (National Center for Health Statistics 2001). Four phthalate metabolites—monoethyl phthalate (MEP), mono-2-ethylhexyl phthalate (MEHP), mono-*n*-butyl phthalate (MBP), and monobenzyl phthalate (MBzP)—were present in more than 75% of U.S. subjects sampled (Blount et al. 2000b; CDC 2001).

Evidence of general population exposure to phthalates (Blount et al. 2000b; CDC 2001), as well as *in vitro* studies suggesting that some phthalates are hormonally active (Harris et al. 1997; Nakai et al. 1999) and animal studies showing associations between some phthalates and testicular toxicity (Gangolli 1982; Li et al. 1998; Parks et al. 2000; Sharpe et al. 1995; Thomas et al. 1982), has generated both public and scientific concern about potential reproductive effects of phthalates. Recent *in vitro* studies using the alkaline comet assay (single-cell gel electrophoresis) found di-*n*-butyl phthalate (DBP) and di-isobutyl phthalate (DiBP) to be genotoxic in human epithelial cells of the upper aerodigestive tract (Kleinsasser et al. 2000a), as well as in mucosal cells and lymphocytes (Kleinsasser et al. 2000b). Additionally, the comet assay was used to detect DNA damage in human lymphocytes induced by *in vitro* exposure to DEHP and MEHP (Anderson et al. 1999).

A lack of consensus on which semen quality tests are the best predictors of human male fertility has led to the development of several new methods to evaluate semen quality. The traditional semen analysis measures sperm concentration, motility, and morphology (World Health Organization 1999). Several laboratory techniques are used to evaluate sperm DNA, such as the sperm chromatin structure assay (SCSA) (Evenson et al. 1991). The SCSA may prove to be a useful clinical test because of its high repeatability and its ability to measure an aspect of fertility that differs from what can be offered by the traditional semen analysis (Evenson et al. 1999). Other DNA tests include fluorescence *in situ* hybridization, used to measure aneuploidy, as well as assays used to measure DNA integrity, including single-cell microgel electrophoresis (comet assay) and the terminal deoxynucleotidyl transferase–mediated dUTP-biotin end-labeling (TUNEL) assay (Lähdetie et al. 1996; Martin 1993; Sun et al. 1997; World Health Organization 1999).

Few published human studies have examined the effect of environmental chemicals on DNA integrity in sperm as measured by the comet assay. In the present study, we used the neutral comet assay to measure DNA integrity in human sperm and investigated whether DNA integrity was associated with urinary concentrations of five phthalate monoesters.

Materials and Methods

Subjects. The study was approved by the Harvard School of Public Health and Massachusetts General Hospital (MGH) Human Subjects Committee, and all subjects signed an informed consent form. Subjects were recruited from an ongoing semen quality

Address correspondence to R. Hauser, Dept. of Environmental Health, Occupational Health Program, Bldg. 1 Rm. 1405, 665 Huntington Ave., Boston, MA 02115-9957 USA. Telephone: (617) 432-3326. Fax: (617) 432-0219. E-mail: rhauser@hohp.harvard.edu

We also thank A. Calafat, Z. Chen, S. Swan, J. Frelich, L. Godfrey-Bailey, A. Larson, N. Mercedat, L. Pothier, A. Trisini, P. Hengel, and Impuls computergestützte Bildanalyse GmbH, Germany.

We acknowledge the National Institute of Environmental Health Sciences grants ES09718, ES00002. NIH training grant T32 ES07069 supported S.M.D.

The authors declare they have no conflict of interest.

Received 6 May 2002; accepted 4 December 2002.